Application of industrial enzymes in the traditional technology of alcohol from cooked sticky rice in Nam Dinh province, Vietnam

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Abstract. Based on the traditional technology of alcohol from cooked sticky rice in Nam Dinh province, Vietnam, the experiments were conducted by the way to kept the technological process as well as the proportion of ingredients. The quantity of the sticky rice for every experiments was 10 kg. The obtained results showed that the addition of 0.05% enzyme SEBflo-TL compared to the dry matter of sticky rice leads to an increase of the absolute anhydrous alcohol by 10%. Combined application of 0.05% enzyme SEBflo-TL and 0.08% SEBrew-GL for hydrolysing starch and β -glucan, the amount of obtained anhydrous alcohol is 4.6 liters compared to 4.0 liters in the sample control without enzyme application, yield increased by 15% and could provide significant economic benefits to wine producer. In other hand, after the first distillation, the fractional distillation reduced concentration of metanol, acetaldehyde and improved volatile compounds of producted alcohol. The finished alcohol met the Vietnam National technical regulation for alcoholic beverages and are unlikely to cause toxicity to consumers.

1 Introduction

In Vietnam and many Asia's countries, rice has been a staple food for humans since ancient times. Homemade alcohol also has a long history. Every year, nearly 300 million liters of handmade alcohol are supplied to the market, more than 70% of the total alcohol production in the country. Homemade alcohol from sticky rice of Nam Dinh province - Vietnam is a specialty produced by experienced artisans, by the characteristic quality of sticky rice, traditional starter culture and water. Most of Nam Dinh cooked sticky rice alcohol is produced in households by small-scale manual methods. Some of this alcohol is not produced according to stable technology and can affect the health of consumers.

Traditional alcohol is produced from white rice and sticky rice, homemade alcohol from sticky rice has a more specific aromatic flavor and is more expensive. Cooked sticky rice has a high amylopectin content, usually at 90-95% of total starch compared to 70-80% in white rice depending on the rice variety. In the traditional technology, the actual conditions for starch hydrolysis in sticky rice are not quite suitable, leading to a lower alcohol recovery rate compared to the normal rice. The hemicellulose component in sticky rice, including β -glucans in the endosperm cell wall, can also be a source of fermentable sugars, capable to form alcohol [7].

State-level projects have been conducted to improve the technological level and quality of homemade alcohol in Vietnam.

"Improving technology, equipment and building an industrial line to produce traditional alcohol" was a production project under the State Science and Technology Program [4]. The project "Research and building technological process and production model of Mai Ha specialty alcohol in Hoa Binh province", in project yeast cake was analyzed quality, research and selection of pure microorganisms with high enzyme capacity and high ethanol production, and also established the yeast cake production process and formed equipments model with capacity of 400 liters/day [3].

Research on the influence of different yeast cakes on the composition of aromatic substances in distilled rice spirit has also been determined by Korean scientists [9]. The influence of different rice varieties on yield, some ingredients and alcohol quality has also been published by Nepal Journal Food Science and Technology [8]. Scientists from Cambodia and the Philippines implemented recovery efficiency improvement by upgrading the sanitary conditions and the techniques of rice cooking, fermentation, and distillation [6].

Industrial enzymes have been guided economic efficiency and widely applied not only in Vietnam, also around in the world. The gelatinization process of rice is usually carried out with an α-amylase preparation, alcohol factories apply glucoamylase to saccharify starch. Breweries use β -glucanase to hydrolyze glucans to reduce the viscosity of the beer wort and improve extraction efficiency. The application of industrial enzymes in traditional rice alcohol production has also been carried out by many of authors. However, research on the

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application of enzyme preparations containing β-glucanase to hydrolyze endosperm cells wall in sticky rice is not found. The present study was conducted to determine a method of industrial enzyme application to improve alcohol recovery compared to the traditional sticky rice alcohol production without using industrial enzyme supplement.

2 Materials and Methods

2.1. Materials

Sticky rice: Using DT21 variety - material in the production of traditional sticky rice alcohol in Nam Dinh. The rice based alcohol must be brown rice and still have thin layer bran on the grain, when the rice is milled, only the outer husk is removed. Rice is straw yellow, cooked rice is glutinous with a characteristic aroma.

Yeast cake (starter culture): Using traditional yeast cake of Nam Dinh province. This yeast cake was used throughout the study.

Water: Using treated soft water obtained by reverse osmosis technology with pH 6.0 - 6.5

Enzyme preparations: The experiments were carried out with two enzymes originated from India: SEBflo-TL (β- glucanase) and SEBrew-GL (glucoamylase). SEBrew-GL is commonly used for saccharification in the industrial production of alcohol, while SEBflo-TL reduces viscosity and shortens filtration time in brewing technology. SEBflo-TL and SEBrew-GL are ISO 9002 compliant. Specifications comply with FAO/WHO food enzyme standards JECFA, FCC and IFOAM.

2.2. Methods

The traditional process of sticky rice alcohol production was identified as the subject of the study and was kept stablein all experiments. The steps include: i/ soak rice for 8 - 10 h with 40 - 45°C hot water, wash and cook rice, ii/ cool cooked rice on stainless steel tray to about 30 -35°C, thickness of cooked rice layer 3 - 4 cm; iii/ inoculation (mixing rice with starter culture): mix yeast cake powder with cooled rice at the rate of 3% compared to raw sticky rice, iv/ Rice solid state fermentation for 3 -4 d until rice has a slight aroma of alcohol, taste sweet and slightly spicy of alcohol. Solid fermentation is carried out under aerobic conditions in a cool place in summer and kept warm in winter, v/ add purified water in the ratio of rice: water 1:3 and the rice liquor fermentation is continued for 7 - 8 d (depending on the ambient temperature) under anaerobic conditions until all the the wort residue settles to the bottom of the jar; vi/ distills to the end of the alcohol to obtain the first alcohol product; vii/ The fractional distillation technique to remove part of the unwanted impurities (methanol, aldehydes, fusel oil, ...) is carried out to obtain the second distilled alcohol by diluting the first distilled alcohol to a concentration of 20% Vol. and remove the first 5% alcohol in the distillation.

The experiments were conducted according to the traditional method of making sticky rice alcohol with a scale of 10 kg of sticky rice/batch, supplemented with enzyme preparations with different ratios (compared to absolute dry matter of sticky rice). After the fermentation and distillation, the experimental samples were determined the concentration of alcohol formed in the fermentation and compared with the control sample without enzymes to determine the appropriate ratio of enzyme preparations.

Based on the recommendations of the enzyme manufacturer and a many of survey experiments.

The experiments were conducted with five samples using SEBflo-TL. The content of additional enzymes SEBflo-TL in the experiments were 0.03, 0.04, 0.05 and 0.06% (dw/dw).

The experiments were conducted with 5 samples using SEBrew-GL. The content of additional enzymes SEBrew-GL (glucoamylase) in the experiments were 0.06, 0.08, 0.1 and 0.12% (dw/dw).

Experiments were conducted three times, obtained data were processed using Microsoft Excel 2013.

2.3 Analytical methods

The absolute dry matter content of sticky rice was determined by drying to constant weight.

Crude protein in raw sticky rice was determined according to the Kjeldahl method.

Composition of crude fiber in glutinous rice was determined according to the ANKOM filter bag method (ANKOM Technology method).

Starch composition in sticky rice was determined by the method of using enzyme amyloglucosidase (gluco amylase).

The alcohol concentration is determined by the pycnometer method.

The composition of aromatic substances was determined by gas chromatography on a Clarus 500 Perkin Elmer instrument.

3 Results and discussion

3.1. Analysis of ingredient sticky rice

The results of the analysis of components of DT21 sticky rice according to the methods described in section 2.3 are presented in Table 1.

According to the Vietnam Food Composition, the protein content in crude sticky rice is 8.6%; in machine sticky rice is 8.4%; in crude rice is 8.1%; in unprocessed rice is 7.9%; in brown rice is 7.5%. Various types of sticky rice contain different proportions of trace elements such as potassium, phosphorus, copper, iron, magnesium, calcium, manganese, zinc, sodium, etc. Sticky rice is rich in vitamins PP, B2, B1 and rice contains almost all amino acids, including non-substituted amino acids [2].

According to the obtained results, the moisture content of sticky rice is 13.6%, which is within the normal range in the food industry.

Table 1. Chemical composition of raw sticky rice

№	Parameter	Unit	Value
1	Moisture	%	13.6
2	Crude protein	%	7.85
3	Crude fiber	%	0.27
4	Starch	%	70.52

The crude fiber content of 0.27% and crude protein of 7.85% is relatively low, which can be assumed to be due to the DT21 sticky rice being milled too well and the protein-rich aleurone layer has been partially removed.

3.2. Research on the method of supplementing enzyme SEBflo-TL

In the traditional technology for the production of sticky rice alcohol, the mold in the yeast cake produces extracellular enzymes to hydrolyze starch and other macromolecular components present in the grain during the solid state fermentation, yeasts convert fermentable sugars to alcohol in the liquor fermentation stage [7].

Alcohol production is carried out by traditional methods described in section 2.2. Evaluation of the cooking process showed that sticky rice was completely gelatinized and facilitated the α-amylase to hydrolyze starch. However, the β-glucan component in the endosperm cell wall may not be completely hydrolyzed, resulting in low ethanol yield and poor wine quality. The results of the experiments on adding the enzyme preparation SEBflo-TL (β-glucanase) to sticky rice are presented in Table 3.2.

Table 2. Effect of enzyme ratio SEBflo-TL on alcohol yield

№	Sample	Ratio SEBflo-TL, %	Alcohol concentration, %Vol	Alcohol volume, L	Anhydrous alcohol, L
1	Control	0.00	40.00 ± 0.15	10	4.00
2	Sample 1	0.03	42.00 ± 0.10	10	4.20
3	Sample 2	0.04	43.30 ± 0.12	10	4.33
4	Sample 3	0.05	44.00 ± 0.08	10	4.40
5	Sample 4	0.06	44.20 ± 0.12	10	4.42

It can be hypothesized that the application of the preparation SEBflo-TL not only catalyzes the hydrolysis of β- glucan, but also releases the organic macromolecular components present in the form of complexes and to improve the hydrolysis of other components of raw material sticky rice.

With adding 0.03% enzyme SEBflo-TL, the alcohol concentration obtained in the experimental sample increased significantly, reaching 42.0% Vol. higher than 40.0% in the control sample without enzymes. Determining the percentage of SEBflo-TL added from 0.04% to 0.06% resulted in an increase in the finished alcohol concentration, however, when the enzyme ratio increased from 0.05% to 0.06%, the effect trivial.

When 0.05% SEBflo-TL was added, with the same volume of finished alcohol, the alcohol concentration increased from 40% to 44% Vol. equivalent to 10% and is a value worthy of attention.

Based on the obtained results, the rate of SEBflo-TL enzyme preparation was selected as 0.05% to carry out the next experiments.

3.3. Research on the method of supplementing enzyme SEBrew-GL

Further experiments were carried out with 0.05% of the enzyme SEBflo-TL and the addition of different ratios of the enzyme SEBrew-GL which is glucoamylase in order to improve the starch hydrolysis efficiency. The method of implementation was described in Section 2.2. The results of the analysis of the alcohol content obtained from the experimental samples are presented in Table 3.

Table 3. Effect of enzyme ratio SEBrew-GL on alcohol yield

№	Sample	Ratio SEBrew-GL, %	Alcohol concentration, %Vol	Alcohol volume, L	Anhydrous alcohol, L
1	Control	0.00	44.00 ± 0.10	10	4.40
2	Sample 1	0.06	45.30 ± 0.12	10	4.53
3	Sample 2	0.08	46.00 ± 0.08	10	4.60
4	Sample 3	0.10	46.10 ± 0.15	10	4.61
5	Sample 4	0.12	46.20 ± 0.10	10	4.62

The enzyme glucoamylase catalyzes the hydrolysis of starch and forms a large amount of fermentable sugars such as glucose and maltose from the non-reducing end of the polysaccharide chain.

With adding 0.06% enzyme SEBrew-GL, the alcohol concentration obtained in the experimental sample increased significantly, reaching 45.3% compared to 44.0% in the control sample without SEBrew-GL. The verifying of the percentage of added SEBrew-GL from 0.06% to 0.08% leaded to an increase in the finished alcohol concentration, however, when the rate of enzyme increased from 0.09% to 0.12%, the effect was no significant.

Based on the results of the conducted experiments and considering the economic efficiency, the selected ratio of SEBrew-GL product was 0.08%, combined with 0.05% SEBflo-TL.

Based on the results of hydrolysis of cooked sticky rice, ethanol fermentation efficiency, and economic efficiency, the ratios of SEBrew-GL and SEBflo-TL were selected for addition to cooked sticky fermentation as 0.08 and 0.05%, respectively. With the combination of 2 enzyme SEBflo-TL and SEBrew-GL, the amount of anhydrous alcohol was 4.6 L compared to 4.0 L in the control without enzyme addition, an increase of 15%, which is significant economy for traditional wine producers.

Traditional producer and assessor have not found any significant difference in the quality of alcohol with enzymes compared with samples without industrial enzymes.

Research on the enzyme application in traditional alcohol production also has been published by other authors. Enzyme preparation Stargen 001 (Genencor) containing Aspergillus kawachi α-amylase enzyme obtained from *Trichoderma reesei* and glucoamylase

from *Aspergillus niger* was applied to improve the degree of starch hydrolysis of sticky rice alcohol production. By the internal α-amylase and the external glucoamylase group, Stargen 001 improves the starch granule hydrolysis efficiency [1].

3.4. Research on the composition of distilled alcohol

With the obtained results, to prepare and carry out the rice solid state fermentation, the selected ratio of SEBrew-GL product was 0.08%, combined with 0.05% SEBflo-TL. In order to improve the alcohol quality as a subject of the research and to create a safe product, in accordance with the national standard of distilled alcohol, the obtained after first destilation alcohol was refined to improve the concentration of alcohol, remove some toxic ingredients such as methanol, acetaldehyde, and at the same time retain many typical aroma and flavors, characteristic of the finised product. The results of the alcohol composition analysis by GC method are shown in Table 4.

Table 4. Composition of distilled alcohol, referred to absolute anhydrous alcohol

№	Compounds	Unit	First distillation	Second distillation
1	Acetaldehydes	mg/L	105.5	91.8
2	Methanol	mg/L	30.8	27.0
3	n-Propanol	mg/L	554.0	514.5
4	Ethyl acetate	mg/L	503.3	480.1
5	Methyl propionate	mg/L	5.2	4.9
6	Iso-butanol	mg/L	576.7	511.0
7	Methyl iso-valerate	mg/L	0.2	0.2
8	3-methyl butanol	mg/L	886.7	812.1
9	2-methyl butanol	mg/L	233.4	205.8
10	Ethyl butyrate	mg/L	0.4	0.3
11	Iso-amyl acetate	mg/L	2.8	2.1
12	Furfuraldehydes	mg/L	2.8	1.4
13	Ethyl octanoate	mg/L	1.1	0.4
14	Phenethyl alcohol	mg/L	6.4	4.1
15	Phenethyl acetate	mg/L	0.1	0.1
16	Ethyl decanoate	mg/L	1.6	1.1

The obtained results of Table 4 showed that the content of volatile substances such as methanol, acetaldehyde and ethyl acetate decreases after second distillation. The small decrease showed that the level of equipment and distillation techniques of the traditional technology cannot be compared with those of industrial distillation scale. These substances have a lower boiling point than ethanol, so they evaporate quickly at the first stage in the second distillation and are discarded.

On second re-distillation, the concentration of ethanol was increased compared to the first distillation. The non-volatile components were recovered in the evaporator and leading to a decreased concentration in finished product.

According to Table 4, many components of the finished alcohol in this study as higher alcohols have similar concentrations to that of rice alcohol produced in Korea [9]. Compared with Kim Son alcohol which is also a famous alcohol product in Vietnam, Nam Dinh alcohol contains a lower amount of methanol and the same level of acetaldehyde [5]. Studies in Phu Tho province - Vietnam showed that 17/20 samples of homemade rice alcohol with concentration of 29 - 58% v/v and contained methanol, the average value of methanol was determined to be 9 mg/L, significantly lower than the alcohol of this study, distilled rice alcohol does not contain methanol content to the point where it can be toxic to consumers [10]. Besides, the methanol content in the alcohol of this study was significantly lower than the requirements according to Vietnam National Technical Regulation for Alcoholic Beverages [11].

4 Conclusion

The results of studies on traditional technology for the production of alcohol from sticky rice in Nam Dinh province - Vietnam have shown that the addition of 0.05% SEBflo-TL leads to about a 10% increase in the absolute anhydrous alcohol content in the finished alcohol. With the combination of 0.05% enzyme preparation SEBflo-TL and 0.08% SEBrew-GL anhydrous alcohol yield was 4.6 liters compared with 4.0 liters in the control sample without enzyme addition, yield 15% increase. By the method of fractional distillation, a part of methanol and acetaldehyde were removed and the product quality was improved. Finished alcohol meets Vietnam National technical regulation for alcoholic beverages and is unlikely to cause toxicity to consumers. The results of studies on improving alcohol quality through long aging in oak materials will be published in the next paper.

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